

Remarks

Enclosed please find a new set of claims, comprising amended claim 12 and newly presented claims 13-18.

Newly presented claims 13 to 18 are based on former claims 2 and 6 to 10. The dependencies have been adapted. Therefore, no new matter has been added.

**Rejection Under 35 U.S.C. § 112, second paragraph**

Claim 12 has been rejected under 35 U.S.C. § 112, second paragraph as being indefinite. In particular, the Examiner alleges that step d) of claim 12 is unclear — stating that the cells must first contain a selection marker, otherwise selection would be impossible. According to Examiner's understanding of the invention, it is imprecise how a selection could occur, since loss of the selection marker precludes the continuation of the selection conditions. Applicant has amended this claim to make it more clear. Withdrawal of this rejection is respectfully requested.

Examiner is correct in stating that the first DNA expression cassette carries a selection marker (a positive-negative selection marker for double selection), which is outlined in step a). Cells containing the selection marker are then selected. Positive selection means that cells, which contain the selection marker, survive the selection conditions. Cells, which do not contain the marker, cannot grow in the selection medium simply because the marker is essential for survival under selection conditions. By doing so, it is ensured that only cells containing the marker are enriched in the medium.

The aim of the invention is to exchange the selection marker, which is flanked by the F and F3 recognition site, against a second cassette, the second cassette carries a gene of interest for introduction into the genome. As the gene of interest is also flanked by the same FLP recognition sites, it is ensured that the gene of interest will be integrated at the same

chromosomal locus at which the selection marker has been sitting before (true exchange). As a major advantage, the second cassette only carries the gene of interest and no further selection marker, which would be considered to be of disadvantage for human gene therapy, as they are artificial sequences.

As a next step, the positive selection conditions are maintained for a period of time, which is sufficiently long to ensure the complete exchange of the first expression cassette against the second expression cassette. If the positive selection conditions were stopped too early, cells not containing the first expression cassette would survive.

To ensure the completeness of the exchange reaction, step f) is included. Negative selection means that only those cells may survive the exchange reaction, which do not contain the selection marker. This means that these cells now contain the second cassette containing the gene of interest. Cells bearing the selection marker (negative selection marker) will die.

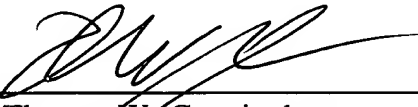
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A check in the amount of \$55 is enclosed to cover the Petition fee of \$55. Please charge any additional fees or credit any overpayments as a result of the filing of this paper to our Deposit Account No. 02-3978 — a duplicate of this paper is enclosed for that purpose.

Respectfully submitted,

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